Familial hypercatabolic hypoproteinemia caused by deficiency of the neonatal Fc receptor, FcRn, due to a mutant β_2-microglobulin gene

Manzoor A. Wani*, Lynn D. Haynes†, Jonghan Kim*, C. L. Bronson*, Chaity Chaudhury*, Sudhasri Mohanty*, Thomas A. Waldmann‡§, John M. Robinson¶, and Clark L. Anderson*‖

Departments of *Internal Medicine and ¶Physiology and Cell Biology, Ohio State University, Columbus, OH 43210; †Department of Surgery, University of Wisconsin, Madison, WI 53792; and ‡Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

Contributed by Thomas A. Waldmann, January 20, 2006

Two siblings, products of a consanguineous marriage, were markedly deficient in both albumin and IgG because of rapid degradation of these proteins, suggesting a lack of the neonatal Fc receptor, FcRn. FcRn is a heterodimeric receptor composed of a nonclassical MHC class I α-chain and β_2-microglobulin (β_2m) that binds two ligands, IgG and albumin, and extends the catabolic half-lives of both. Eight relatives of the siblings were moderately IgG-deficient. From sera archived for 35 years, we sequenced the two siblings' genes for the heterodimeric FcRn. We found that, although the α-chain gene sequences of the siblings were normal, the β_2m genes contained a single nucleotide transversion that would mutate a conserved alanine to proline at the midpoint of the signal sequence. Concentrations of soluble β_2m and HLA in the siblings' sera were <1% of normal. Transfection assays of β_2m-deficient cultured cells with β_2m cDNA indicated that the mutant β_2m supported <20% of normal expression of β_2m, MHC class I, and FcRn proteins. We concluded that a β_2m gene mutation underlies the hypercatabolism and reduced serum levels of albumin and IgG in the two siblings with familial hypercatabolic hypoproteinemia. This experiment of nature affirms our hypothesis that FcRn binds IgG and albumin, salvages both from a degradative fate, and maintains their physiologic concentrations.

albumin | IgG | MHC class I | hypoalbuminemia | hypogammaglobulinemia

The neonatal Fc receptor, FcRn, is a heterodimer of a nonclassical MHC class I (MHC I) α-chain and β_2-microglobulin (β_2m) that binds the two most abundant serum proteins, IgG (1) and albumin (2), after their constitutive uptake by many cells of the body. FcRn binds both ligands with high affinity at the low pH of acid endosomes and releases them at the physiologic pH of the cell surface, where they are free to circulate, thus diverting them from lysosomal degradation (2–5). Such FcRn-mediated recycling explains the uniquely long half-lives and the direct concentration–catabolism effect of IgG and albumin. The capacity of this salvage mechanism is astonishing; without FcRn, mice would need dramatically increased albumin and IgG synthetic rates to maintain normal concentrations of these proteins (6).

In accordance with this hypothesis, we have found that mice lacking FcRn because of defective genes for the α-chain or β_2m show low serum concentrations and rapid degradation rates of both IgG and albumin (2). Searching for analogous FcRn-deficient humans, we reexamined two siblings, born of a consanguineous marriage, whose disorder, familial hypercatabolic hypoproteinemia (FHH), had been thoroughly studied in the late 1960s (8, **). Both siblings manifested hypercatabolic albumin and IgG deficiencies for which no known cause could be found at the time of investigation. Recognizing that these two individuals might have been homozygous for a defect in one or the other of the two genes encoding the heterodimeric FcRn, we analyzed their archived sera, the subjects themselves being no longer available. We now ascribe their defect to a severe FcRn deficiency resulting from a β_2m gene mutation.

Results

Analyzing serum samples by ELISA, we found soluble β_2m (sβ_2m) concentrations for the two siblings, S1 and S2, to be <10 ng/ml (Table 1). These values were ≈100-fold less than those for our three control groups, which included three normal sera (N1, N2, and N3) analyzed simultaneously, eight National Institutes of Health (NIH)-archived sera analyzed simultaneously, and assay normal values. The serum concentrations of soluble HLA (sHLA) were likewise very low, being <0.2% of the normal mean (Table 1). Values for serum iron, total iron-binding capacity (TIBC), percent transferrin saturation with iron (%TS), and ferritin, determined either in our laboratories or at NIH in the 1960s, were normal (Table 1).

We PCR-amplified and sequenced all exons of both the β_2m and the FcRn α-chain genes from DNA purified from sera of the two siblings and three normal individuals. Whereas gene sequences in both directions of β_2m exons 2, 3, and 4 of all individuals were normal, the sequences of exon 1 of β_2m in the two siblings alone showed a single nucleotide transversion (G913C) (Fig. 1*a*). This mutation was confirmed by digesting the amplified exon 1 DNA with a restriction enzyme that uniquely distinguishes between the normal and the mutant nucleotide at this site (Fig. 1*c*). The pattern of bands of digested DNA from the two siblings was distinctly different from those of the normal individuals (Fig. 1*b*). The G913C transversion would be predicted to cause a mutation from alanine to proline at amino acid 11 at the midpoint of the signal sequence (Fig. 1*d*). We estimated the frequency of this G913C mutation in an ancestry-matched population by assessing a collection of 100 DNA samples with the restriction enzyme digestion assay described in Fig. 1*c* and found no other instances of such a mutation. Therefore, the gene frequency of this mutation in the sampled population is <0.5%. The sequences of the gene for the FcRn α-chain in the two siblings were normal.

To test the prediction that the mutant β_2m signal sequence would hinder cellular expression of β_2m protein, we transfected a β_2m-lacking human cell line with a plasmid containing mutant β_2m cDNA bearing the single nucleotide transversion (mut),

Conflict of interest statement: No conflicts declared.

Abbreviations: FcRn, neonatal Fc receptor; β_2m, β_2-microglobulin; TIBC, total iron-binding capacity; %TS, percent transferrin saturation with iron; FHH, familial hypercatabolic hypoproteinemia; MFI, mean fluorescence intensity; wt, wild type; mut, mutant; MHC I, MHC class I; sHLA, soluble HLA.

§To whom correspondence may be addressed. E-mail: tawald@helix.nih.gov.

‖To whom correspondence may be addressed at: 415 HLRI, 473 West Twelfth Avenue, Columbus, OH 43210. E-mail: anderson.48@osu.edu.

** Waldmann, T. A., Miller, E. J. & Terry, W. D. (1968) *Clin. Res.* **16,** 45 (abstr.).

www.pnas.org/cgi/doi/10.1073/pnas.0600548103

Table 1. Serum components of the two FHH siblings and controls

Serum tested	Serum component					
	sβ_2m, μg/liter	sHLA, μg/liter	Iron, μg/liter	TIBC, μg/liter	%TS	Ferritin, μg/liter
FHH siblings						
S1	9	16	8, 8*	40, 27*	21, 29*	5
S2	7	2	7, 4*	32, 28*	13, 15*	6
Controls						
Normal human sera	1,180 ± 126	838 ± 536	ND	ND	ND	ND
Archived sera	1,300 ± 1,000	788 ± 793	ND	ND	ND	ND
Laboratory standard values	800–2,000	1,600 ± 1,000	7–18	25–45	26–38	3–30

Sera from the two siblings, S1 and S2, along with three normal human sera and eight sera archived at NIH since the 1960s, were tested for sβ_2m, sHLA, serum iron, TIBC, %TS, and ferritin, and they were compared with laboratory standard values for each assay, given as either normal range or mean ± SD. The sβ_2m and sHLA values for S1 and S2 are the means of two determinations, with deviations from the mean being <10%. S2 values have been corrected as described in *Materials and Methods.* ND, not determined.
*Values extracted from NIH medical records from the 1960s.

with wild-type (wt) β_2m cDNA, and with the empty plasmid vector (p). We then assessed cell surface expression of β_2m protein by flow cytometry by using a fluorescent antibody specific for β_2m. The fluorescence profiles shown in Fig. 2*a* indicate that cells transfected with wt β_2m cDNA were on average brighter [mean fluorescence intensity (MFI) = 109] than control cells (MFI = 4), and that a broad shoulder of very bright cells was apparent (peak of 20% of mut transfected cells at a fluorescence intensity of ≈450). Cells transfected with empty plasmid were not distinguishable from cells incubated with an irrelevant antibody of the same isotype as the anti-β_2m antibody (Fig. 2*c*), which was consistent with the cells being β_2m-deficient. Cells transfected with mut β_2m and stained with anti-β_2m antibody (MFI = 15) were only 10% as bright as cells transfected with wt β_2m cDNA. An equivalent conclusion was reached if only cells brighter than the vector-transfected cells were analyzed; i.e., mut β_2m-transfected cells were 6% as bright as wt β_2m-transfected cells.

Anticipating that β_2m expression would rescue the surface expression of MHC I in this β_2m-deficient cell line, we also

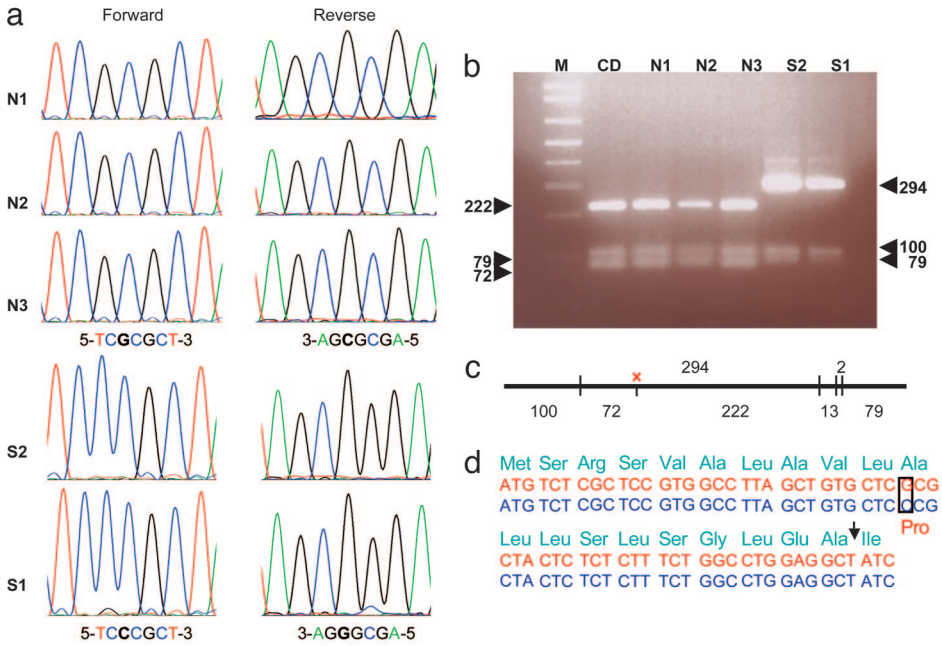

Fig. 1. The β_2m gene mutation in FHH patients. (*a*) Electropherograms comparing the nucleotide sequences of β_2m exon 1 DNA amplified from serum samples of three normal individuals (N1, N2, and N3) and the two patients (S1 and S2). The emboldened base in the forward sequences identifies the single nucleotide mutation from G to C in the two FHH patients. The reverse strand sequence complements the forward sequence. (*b*) An ethidium-stained separation gel showing HinP1I (G/CGC) restriction enzyme digests of a 488-bp segment of exon 1 DNA sequence of the β_2m gene PCR-amplified from relevant DNA samples. Marginal numbers indicate fragment sizes in bp. DNA from three normal sera and from cells of one normal individual (CD) show a wt restriction pattern with six restriction fragments (2, 13, 72, 79, 100, and 222 bp), whereas DNA from the patients show a diagnostically distinctive pattern of five restriction fragments (2, 13, 79,100, and 294 bp). (*c*) Restriction map of the PCR-amplified 488-bp segment. The vertical lines denote the HinP1I restriction sites; the numbers indicate the resulting fragment sizes. The red x marks the mutated (and thereby lost) HinP1I restriction site in the DNA of the two FHH patients, surrounding the point mutation (GCGC/CCGC) and causing the loss of the 72-bp fragment and the gain of the 294-bp fragment. (*d*) The 20-aa signal sequence (to arrow) of the β_2m protein, with corresponding codons. The box indicates the single nucleotide transversion from G in the normal sequence (red) to C in the FHH patient sequence (blue). This transversion results in a single amino acid mutation from alanine to proline.

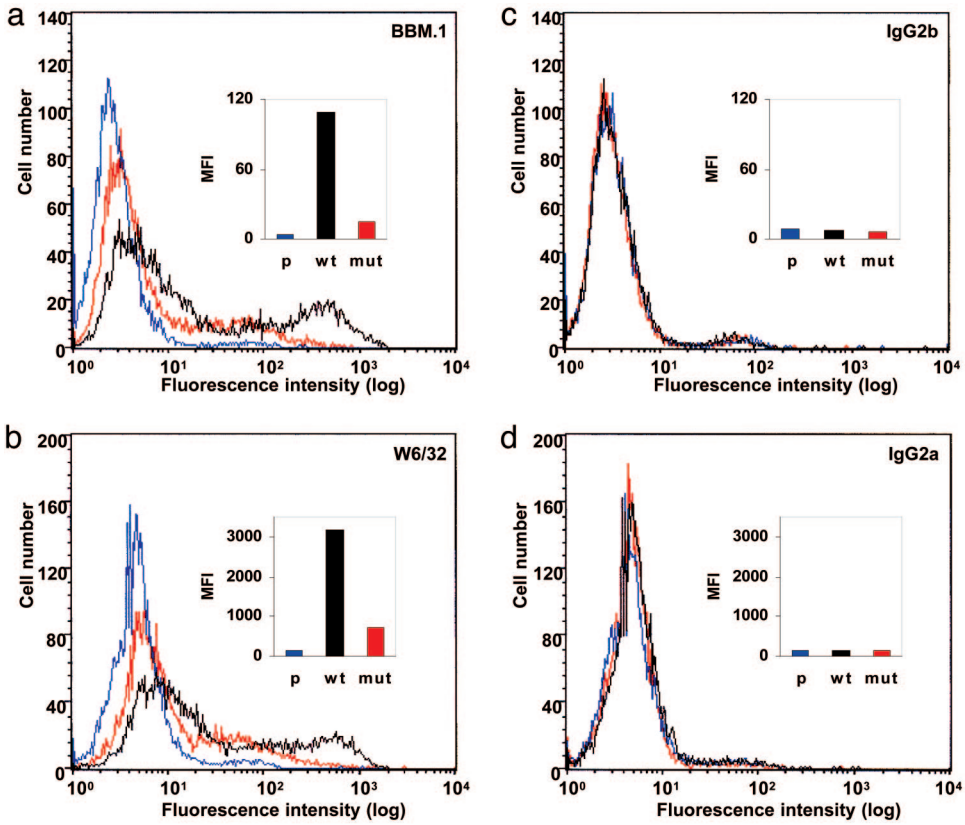

Fig. 2. Overexpression of FHH mutant β_2m fails to restore MHC I and β_2m expression in a β_2m-deficient cell line. A β_2m-deficient cell line was transfected with a plasmid containing wt hβ_2m cDNA (black), FHH mutant hβ_2m cDNA (red), or plasmid alone (blue). Cells were stained with anti-β_2m antibody (*a*), with anti-MHC I antibody (*b*), and with IgG isotype-matched control antibodies, followed by FITC-labeled goat anti-mouse secondary antibody (*c* and *d*). The results of one of the three representative flow cytometry experiments are expressed as histograms of fluorescence intensity (log scale) vs. the number of cells.

measured the binding of an MHC I-specific antibody in parallel flow cytometric experiments (Fig. 2 *b* and *d*). Cells transfected with wt β_2m cDNA showed high levels of MHC I expression (MFI = 3,176); those transfected with empty vector (MFI = 156) showed no expression beyond background levels; and those transfected with mut β_2m cDNA (MFI = 738) showed levels of expression <20% of that seen in the wt (duplicate experiments, 19% and 13%). Equivalent results were seen when only those cells brighter than vector-transfected cells were analyzed; i.e., mut β_2m-transfected cells were 20% as bright as wt β_2m-transfected cells.

To measure total cellular, rather than simply surface, expression of mut β_2m, we subjected these same transfected cell preparations to an alternative analysis, namely, immunoblotting of detergent lysates of cells. Analyzing cell lysates directly, we found virtually no mut β_2m expression, whereas cells transfected with wt β_2m expressed considerable protein (Fig. 3*a*, lanes 2 and 3). First immunoadsorbing with anti-β_2m mAb, and then analyzing the immunoadsorbates by immunoblotting with anti-β_2m polyclonal antibody, we found mutant β_2m expression to be evident but minimal (<10% of wt β_2m) (Fig. 3*b*, lanes 2 and 3).

We also measured the capacity of mutant β_2m to enhance the expression of the FcRn heterodimer. Assessing cell lysates by immunoblot with anti-FcRn α-chain antibody, we noted that very little FcRn α-chain was expressed, even after transfection with wt β_2m (Fig. 3*a*, lane 2). We therefore transfected the cells with FcRn α-chain cDNA and noted α-chain protein expression (Fig. 3*a*, lane 4) in the absence of β_2m, as others have reported (9). Cotransfecting these cells with β_2m cDNA, we noted that mut β_2m was only 22% as effective as wt β_2m in enhancing the amount of FcRn α-chain seen in immunoblots (average of two experiments, 14% and 30%) (Fig. 3*a*, lanes 5 and 6 vs. 4). Determination of whether diminished expression of mut β_2m protein resulted from defective transcription or translation was beyond the scope of the present study.

Discussion

Our analysis of the archived serum from these two consanguineous siblings with FHH indicated that both were homozygous for a single nucleotide transversion of the β_2m gene that encoded a mutation from an evolutionarily conserved alanine to a proline in the center of the hydrophobic core of the β_2m signal sequence α-helix. A proline at this site would be expected to disrupt targeting of the nascent polypeptide to the endoplasmic reticulum (10). Indeed, the serum β_2m concentrations in the FHH siblings were very low, and the mutant β_2m cDNA was able to support only 10–20% of wt β_2m expression in a cell transfection assay.

It is clear that a proline mutant might compromise signal peptide function, depending on where it is situated in the sequence. The mutation of alanine to proline does not affect the net charge or hydrophobicity of the α-helix but presumably disturbs the secondary structure as a result of its helix-breaking properties, thus leading to compromised interaction with the signal recognition protein and abnormal insertion into the endoplasmic reticulum membrane (11). Similar proline mutations in signal sequences have been described in specific diseases (12–15).

Although no β_2m gene mutations abrogating protein expression have been described in humans, some cancer cell lines bear

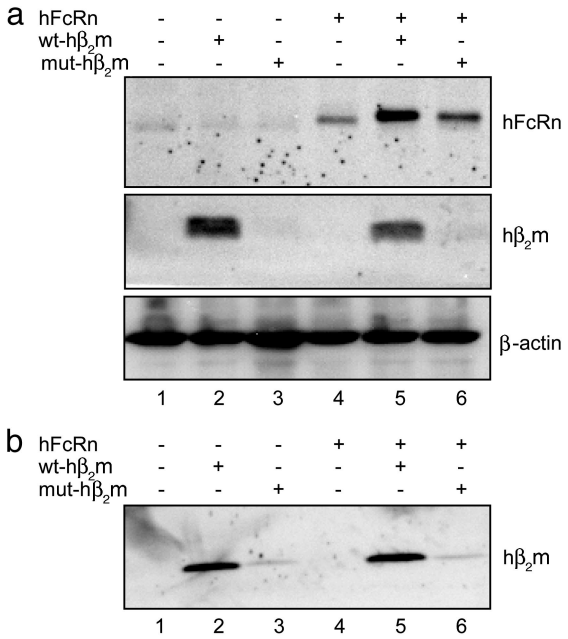

Fig. 3. Mutant β_2m fails to enhance FcRn expression. (*a*) Analysis of lysates. Nonionic detergent lysates of β_2m-deficient cultured cells (FO-1) transfected with tabulated combinations of wt hβ_2m cDNA, mut hβ_2m cDNA, FcRn α-chain cDNA, and vectors alone (all lanes 1), were analyzed by SDS/PAGE and immunoblotting with rabbit anti-human FcRn antibody, rabbit anti-human β_2m antibody, and anti-β-actin antibody as a protein-loading control. (*b*) Immunoadsorption of β_2m. Lysates in OG buffer (pH 7.5) were immunoadsorbed with anti-hβ_2m mAb (BBM.1). Eluted proteins were immunoblotted with rabbit anti-hβ_2m antibody.

null β_2m genes (16). Most of these mutations have been cytoplasmic tail (CT) deletions in the leader sequence that result in reading frame shifts and downstream nonsense mutations. The only known substitution mutation is in the Daudi B cell line, where methionine is replaced by isoleucine as the N-terminal amino acid, abolishing β_2m expression.

β_2m is generally known to be required for efficient expression and function of several classical and nonclassical members of the MHC I family of proteins, which are heterodimers consisting of β_2m and a distinguishing α-chain of about 43 kDa. The family members include FcRn, CD1, hemochromatosis protein (HFE), and MHC I itself. Considering the data available on the two siblings studied, we can conclude with virtual certainty that they were FcRn-deficient. Their phenotype, which features low serum IgG and albumin concentrations and hypercatabolism of both proteins, is mirrored by the β_2m and FcRn α-chain knockout mouse strains (2). Unfortunately, we have no means of assessing FcRn expression directly in these siblings because we have available neither tissue nor a satisfactory assay of serum-soluble FcRn. However, the mutant β_2m cDNA, transfected into β_2m-deficient cells, was severely deficient in supporting the expression of FcRn. Moreover, sHLA concentrations in serum were very low in both siblings, and in a cell transfection assay, mut β_2m cDNA was able to rescue only $\approx$20% of normal surface expression of MHC I protein. Thus, these siblings were likely severely MHC I-deficient. Although they were probably also HFE-deficient, they showed no increase in saturation of transferrin with iron, perhaps because of their relative youth (34 and 17 years of age) or the low penetrance of the type 1 hemochromatosis genotype.

Concluding that the two FHH siblings were homozygous for a defective β_2m allele, we then reassessed the remainder of the pedigree for whom serum IgG concentrations were available (table 1 in ref. 8). On the basis of the β_2m genotype of the two siblings, we inferred that six of eight of the studied relatives were definitely heterozygous, and two were likely heterozygous, for the β_2m gene (patients 5 and 6, the unaffected brother and sister of S1 and S2, had a 66% chance of being heterozygous). We noted that the IgG concentrations of these eight relatives (mean $\pm$ SD = 9.2 $\pm$ 1.4 mg/ml; $P < 0.001$) were distinctly lower than the normal value of 12.4 $\pm$ 2.2 mg/ml. Although we know nothing about the albumin concentrations in the eight relatives, the IgA, IgM, IgD, and κ-chain concentrations were not different from normal. A single normal β_2m allele thus may confer a moderate IgG deficiency. It is unclear whether mice expressing a single β_2m allele are IgG-deficient, although the one large published study of heterozygous β_2m knockout mice concluded that there were no gross differences in IgG concentrations between wt and heterozygotes (17). Nevertheless, β_2m heterozygote mice expressed reduced levels of splenocyte MHC I and had diminished numbers of CD8-expressing thymocytes (18).

On the basis of our analysis of 35-year-old archived sera, we conclude that FcRn deficiency caused by a mutant β_2m gene underlies the hypercatabolism of both IgG and albumin seen in FHH.

Materials and Methods

Cell Line. The β_2m-lacking human melanoma cell line FO-1 (19), kindly provided by William Carson (Ohio State University, Columbus) was maintained in RPMI 1640 medium (Invitrogen) supplemented with 10% heat-inactivated FCS in a 5% CO_2-humidified atmosphere at 37°C.

Serum Samples. Sera from S1 and S2 and from eight anonymous NIH patients had been archived in NIH freezers since the late 1960s. Neither the patients nor tissue were available. The S2 serum used for protein determinations was found to have been concentrated 1.6-fold (calculated by measurement of electrolytes), likely because of sublimation in storage. The results given in Table 1 have, therefore, been corrected by dividing the observed values by 1.6. Additional sera were collected from three healthy adult individuals.

β_2m Serum Concentrations. The β_2m concentrations of the serum samples were determined by using a sandwich ELISA (β_2m ELISA Kit; Alpha Diagnostic, San Antonio, TX). Briefly, 100 μl of diluted serum of the normal individuals (1:100 dilution) and the two FHH patients (1:5 dilution) were loaded in duplicate wells in a 96-well plate coated with anti-human β_2m (hβ_2m) monoclonal antibody and incubated at room temperature for 30 min. The wells were washed five times with wash buffer, and 100 μl of horseradish peroxidase-conjugated rabbit polyclonal anti-hβ_2m antibody was added into each well. The plate was further incubated at room temperature for 30 min. After five washes with wash buffer, 200 μl of substrate solution mix was added into the wells. The reaction was stopped by adding 50 μl of stop solution (0.5 M sulfuric acid), and the yellow color developed was read within 30 min at 450 nm by using an EL_X 808 ELISA plate reader (Biotek Instruments, Luton, U.K.). A reference β_2m standard ranging from 1.25 to 50 ng/ml was run in parallel with each experiment. Normal values for this assay, according to the supplier, are given in Table 1.

Measurement of Iron, Ferritin, and Transferrin. Serum iron and TIBC were measured in the clinical laboratory of the Columbus Children's Hospital by using the pyridyl azo dye ascorbic acid method for iron, a competitive immunoassay for ferritin, and the alumina adsorption method for TIBC. %TS was calculated as 100 $\times$ serum iron concentration/TIBC. Normal values in Table 1 are from the same laboratory. Values at the time of the FHH siblings' NIH evaluation were taken from the hospital record.

ELISA for sHLA. ELISA for sHLA was performed as described (20). Briefly, mAb TP25–99 (a kind gift from SangStat Medical Corporation, Menlo Park, CA), which recognizes both β_2m-free and associated HLA (21), was used as the capture antibody; rabbit anti-human β_2m (Accurate Chemical & Scientific, Westbury, NY) and horseradish peroxidase-coupled goat anti-rabbit IgG (Sigma) were used as the detection antibodies. A standard curve was generated with purified HLA-B7 diluted serially to concentrations between 20 and 0.3125 ng/ml. Tetramethylbenzidine microplate substrate and stop solution (Kirkegaard & Perry Laboratories) were used. The plates were read at 450 nm by using the Bio-Tek ELx800 reader, and data were analyzed with KC3 software (Biotek Instruments). Values for sera of 27 normal individuals and the 8 archived NIH sera are given in Table 1.

Serum DNA Extraction, PCR Amplification, and Mutational Analysis. DNA was extracted from 400 μl of serum by adsorption to silica-gel membrane (QIAamp DNA Blood Mini Kit; Qiagen, Valencia, CA) and quantified with the PicoGreen dsDNA quantitation method by fluorometry (Molecular Probes). PCR was performed in 50-μl reactions containing 2.5 units of TaqDNA polymerase (Qiagen), 1× buffer and solution Q, 2 mM $MgCl_2$, 0.4 μm dNTP mix, 200 nM of each primer, and 25 ng of serum DNA as template. Samples were subjected to PCR conditions as follows: initial denaturation at 95°C for 4 min, followed by 35 cycles of 94°C for 1 min, annealing at 57°C for 1 min, extension at 72°C for 1 min, and a final extension cycle at 72°C for 10 min. A positive cellular DNA and a negative template-free control were also run in parallel with each PCR run. After amplification, the DNA was resolved by electrophoresis through 1.5% agarose gels containing 0.5 μg/ml ethidium bromide, and was visualized on a UV transilluminator and compared with molecular weight markers run in parallel. Single specific bands observed in all PCRs were excised from the agarose gel and purified on QiaexII gel extraction beads (Qiagen), eluted in water, and sequenced with a 3730 DNA Analyzer (Applied Biosystems). All exonic fragments were amplified individually and sequenced one at a time. Primer pairs were chosen from the sequence at least 100 bp upstream of the exon start and at least 130 bp downstream from the end of exon, to ensure analysis of the junctions. Although normal, the sequences of S2 and one normal control individual (N3) contained a single copy of a known allotypic polymorphism, a synonymous C2585T (22) change encoding arginine-171 like the normal allele (23) (GenBank accession no. AF200219; data not shown).

Plasmids and Site-Directed Mutagenesis of hβ_2m cDNA. We used expression vectors pCMV-Sport 6 (cDNA clone MGC:45276; Open Biosystems, Huntsville, AL) containing full-length hβ_2m cDNA (24) (GenBank accession no. BC032589), and pREP9 (Invitrogen) containing full-length human FcRn α-chain cDNA (23) (GenBank accession no. U12255). FHH mutant cDNA was prepared by site-directed mutagenesis (Quik Change Kit; Stratagene), with pCMV-Sport6 β_2m cDNA as the template. The upper oligonucleotide, 5′-GCCTTAGCTGTGCTC*CC*GCTACTCTCTCTTTCTGG-3′, and the lower oligonucleotide, 5′-CCAGAAAGAGAGAGTAGCG*G*GAGCACAGCTAAGGCC-3′, were used for mutation of alanine-11 to proline in the exon 1 signal sequence of the β_2m cDNA (base substitutions italicized). The generated mutant FHH cDNA was sequenced in both directions to ensure fidelity.

Cell Transfection. FO-1 cells were transfected with pCMV-Sport6 containing full-length human β_2m cDNA, FHH mutant β_2m cDNA, and pREP9 (Invitrogen) containing full-length FcRn α-chain cDNA by using Lipofectamine 2000 (Invitrogen) in accordance with instructions from the supplier. Briefly, cells were plated on 10-cm dishes (Corning) overnight in antibiotic-free medium. The next day, 90% confluent cells were washed twice with serum-free medium and overlayed with 3 ml of serum-free and antibiotic-free medium containing 20 μg of each plasmid and 60 μl Lipofectamine, and then with 7 additional ml of medium. After 5 h of culture at 37°C, an additional 10 ml of antibiotic-free medium containing 20% FCS (10% final) was added. Cells were harvested at 24 h after transfection.

Flow Cytometry. Cells were detached from the 10-cm dishes by adding 1 ml of cell dissociation solution (Sigma), and were centrifuged at 400 × *g* for 5 min at 4°C. Cells (1×10^6) were incubated for 1 h at 4°C with 5 μg/ml of primary antibodies, mAb W6/32 for MHC I expression, or BBM.1 for β_2m expression (in 200 μl of PBS containing 0.2% BSA and 0.1% sodium azide). Cells were washed twice and incubated for 1 h with FITC-tagged goat anti-mouse IgG (Caltag, South San Francisco, CA). Cells were then washed three times and resuspended in 0.5 ml of PBS. Control staining with isotype-matched IgG2a and IgG2b proteins was performed for each analysis. Samples were analyzed on a FACS Caliber flow cytometer (Becton Dickinson). The MFIs for all cells stained with each antibody were compared directly. The expression efficiency ($\%E$) of the mutant β_2m compared with the wt β_2m was calculated by subtracting the MFI of the cells transfected with empty vector (e) from the MFI of cells transfected with β_2m and expressing the quotient of specific mut β_2m MFI (m) over specific wt β_2m MFI (w) as a percentage; that is, $\%E = 100(m - e)/(w - e)$.

Immunoblotting. To detect FcRn and β_2m protein, transfected cells were collected and lysed in 0.15 M phosphate buffer, pH 6.0, containing 60 mM *n*-octyl β-D glucopyranoside (OG), 10 mM gluconic acid lactone, 1 mM EDTA, and a mixture of protease inhibitors (OG buffer), as described in ref. 25. Equal amounts of total protein, as determined by bicinchoninic acid assay (Pierce), from all transfections were electrophoresed on a 12% polyacrylamide gel in SDS. Proteins were transferred onto a nitrocellulose membrane by wet-blotting at 150 mA for 2 h. Membranes were blocked with 5% milk in Tris buffered saline/0.05% Tween 20 (TBST) for 1 h, followed by incubation with affinity-purified rabbit polyclonal antibody directed against the CT of the α-chain of human FcRn derived from immunizations with a GST fusion protein of CT [anti-CT(GST)] (1:1,000), or with rabbit polyclonal anti-β_2m antibody (FL-119, 1:1,000 Santa Cruz Biotechnology) at 4°C overnight. After three washes with TBST, the membranes were incubated with secondary horseradish peroxidase-labeled donkey anti-rabbit IgG (1:5,000). Proteins were visualized by enhanced chemiluminescence and were quantified by densitometry with a Fluor-S-Max multiimager using QUANTITY ONE software (Bio-Rad) [except that Hyperfilm (Amersham Biosciences) was used for β-actin].

Immunoadsorption. Cells expressing human β_2m wt cDNA or FHH mutant cDNA were lysed as described above for immunoblotting. Equal amounts of total protein cell lysate were immunoadsorbed with 1 μg/ml BBM.1 and Protein-A agarose (Invitrogen) overnight at 4°C, as described (7). The agarose complexes were washed five times with the OG buffer, and the bound proteins were eluted in SDS sample buffer containing 2-mercaptoethanol by boiling for 10 min. Eluted proteins were separated on a 14% polyacrylamide gel in SDS and transferred to a nitrocellulose membrane. The membrane was blocked with 5% milk and blotted with rabbit anti-β_2m polyclonal antibody, as described (2).

Frequency of the FHH Mutation. We amplified exon 1 of β_2m from cellular genomic DNA of 100 control individuals of north

European descent and from serum genomic DNA of the two affected siblings of British ancestry by using the forward primer 5′-TGAAGTCCTAGAATGAACGCCC-3′ and reverse primer 5′-CGCCCTGAACTTTGTCCCG-3′, in accordance with the conditions described above. The 488-bp PCR-amplified fragment was purified by using a Montage PCR centrifugal filter (Millipore) and eluted in 20 μl of water. DNA was digested at 37°C overnight with 10 units of restriction enzyme HinP1I (New England Biolabs) that recognizes five GCGC sequences within the 488-bp PCR-amplified fragment, one of which is the site of the FHH mutation. The digested DNA was resolved on a 2% ethidium bromide-stained agarose gel and visualized on a UV transilluminator.

We thank Arthur Burghes for valuable advice and for the donation of the DNA samples, Daniel Lui for developing the serum FcRn assay, and Qianzheng Zhu for helpful comments. This work was supported in part by National Institutes of Health (NIH) Grants HD38764, CA88053, and AI57530 and by the Intramural Research Program of the National Cancer Institute (NIH).

1. Simister, N. E. & Mostov, K. E. (1989) *Nature* **337,** 184–187.
2. Chaudhury, C., Mehnaz, S., Robinson, J. M., Hayton, W. L., Pearl, D. K., Roopenian, D. C. & Anderson, C. L. (2003) *J. Exp. Med.* **197,** 315–322.
3. Ghetie, V., Hubbard, J. G., Kim, J. K., Tsen, M. F., Lee, Y. & Ward, E. S. (1996) *Eur. J. Immunol.* **26,** 690–696.
4. Junghans, R. P. & Anderson, C. L. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 5512–5516.
5. Israel, E. J., Wilsker, D. F., Hayes, K. C., Schoenfeld, D. & Simister, N. E. (1996) *Immunology* **89,** 573–578.
6. Kim, J., Bronson, C. L., Hayton, W. L., Radmacher, M. D., Roopenian, D. C., Robinson, J. M. & Anderson, C. L. (2006) *Am. J Physiol.* **290,** G352–G360.
7. Tridandapani, S., Pradhan, M., LaDine, J. R., Garber, S. A. & Anderson, C. L. (1999) *J. Immunol.* **162,** 1408–1414.
8. Waldmann, T. A. & Terry, W. D. (1990) *J. Clin. Invest.* **86,** 2093–2098.
9. Praetor, A. & Hunziker, W. (2002) *J. Cell Sci.* **115,** 2389–2397.
10. Sansom, M. S. P. & Weinstein, H. (2000) *Trends Pharmacol. Sci.* **21,** 445–451.
11. Ryan, P. & Edwards, C. O. (1995) *J. Biol. Chem.* **270,** 27876–27879.
12. Ozawa, T., Takikawa, Y., Nhya, K., Ejiri, N., Suzuki, K., Sato, S. & Sakuragawa, N. (1998) *Br. J. Haematol.* **101,** 47–49.
13. Lanza, F., De la Salle, C., Baas, M.-J., Schwartz, A., Boval, B., Cazenave, J.-P. & Caen, J. P. (2002) *Br. J. Haematol.* **118,** 260–266.
14. Kallio, J., Pesonen, U., Kaipo, K., Karvonen, M. K., Jaakkola, U., Heinonen, O. J., Uusitupa, M. I. J. & Koulo, M. (2001) *FASEB J.* **15,** 1242–1244.
15. Karvonen, M. K., Pesonen, U., Koulo, M., Niskanen, L., Laakso, M., Rissanen, A., Dekker, J. M., Hart, L. M., Valve, R. & Uusitupa, M. I. J. (1998) *Nature* **4,** 1434–1437.
16. Bicknell, D. C., Rowan, A. & Bodmer, W. F. (1994) *Genetics* **91,** 4751–4755.
17. Israel, E. J., Patel, V. K., Taylor, S. F., Marshak-Rothstein, A. & Simister, N. E. (1995) *J. Immunol.* **154,** 6246–6251.
18. Koller, B. H., Marrack, P., Kappler, O. & Smithies, O. (1990) *Science* **248,** 1227–1230.
19. D'Urso, M. C., Want, Z., Cao, Y., Tatake, R., Zeff, R. A. & Ferrone, S. (1991) *J. Clin. Invest.* **87,** 284–292.
20. DeVito-Haynes, L. D., Bushkin, D. S., Bushkin, Y. & Burlingham, W. J. (1998) *Hum. Immunol.* **59,** 426–434.
21. Tanabe, M., Sekimata, M., Ferrone, S. & Takiguchi, M. (1992) *J. Immunol.* **148,** 1302–1309.
22. Gunraj, C. A., Fernandes, B. J. & Denomme, G. A. (2002) *Immunogenetics* **54,** 139–140.
23. Story, C. M., Mikulska, J. E. & Simister, N. E. (1994) *J. Exp. Med.* **180,** 2377–2381.
24. Strausberg, R. L., Feingold, E. A., Grouse, L. H., Derge, J. G., Klausner, R. D. & Collins, F. S. (2002) *Proc. Natl. Acad. Sci. USA* **99,** 16899–16903.
25. Leach, J. L., Sedmak, D. D., Osborne, J. M., Rahill, B., Lairmore, M. D. & Anderson, C. L. (1996) *J. Immunol.* **157,** 3317–3322.